(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/17344 A2

(51) International Patent Classification[7]: A01K 67/027

(21) International Application Number: PCT/CZ00/00064

(22) International Filing Date:
8 September 2000 (08.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PV 3186-99 8 September 1999 (08.09.1999) CZ

(71) Applicant *(for all designated States except US)*: **BIOPHARM, VÝZKUMNÝ ÚSTAV BIOFARMACIE A VETERINÁRNÍ CH LÉČIV A.S.** [CZ/CZ]; Pohoří-Chotouň, 254 49 Jílové u Prahy (CZ).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **TREFIL, Pavel** [CZ/CZ]; K Netlukám 962, 104 00 Praha 10 (CZ). **KOTRBOVA, Alena** [CZ/CZ]; Peškova 729, 341 01 Horaždovice (CZ).

(74) Agent: **PATENTSERVIS PRAHA A.S.**; Jívenská 1/1273, 140 21 Praha 4 (CZ).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
Without international search report and to be republished upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/17344 A2

(54) Title: A METHOD OF TRANSGENIC FOWL CONSTRUCTION

(57) Abstract: A method of transgenic fowl construction using germline spermatogonial cells for transfer of genetic information in fowl strain, which method is carried out so that the testicles only of an acceptor cock are irradiated with gamma rays up to the absorbed dose 8 Gy in one irradiation repeatedly and externally. Thereby, the original germline spermatogonial cells in the testicles of the acceptor cock are destroyed, which acceptor cock is then not able to produce sperms, whereby, the testicles structure, including the Sertoli's and Leydig's cells remains preserved for implantation of foreign germline spermatogonial cells of a donor cock. The new implanted germline spermatogonial cells then continue in production of sperms. The sperms are able to fertilize and that store all genetic information of the germline spermatogonial cells of the donor cock. The implantation is carried out after 50 days and more after the last irradiation. Production of transgenic fowl by this method is easy, because the spermatogonial cells of the donor cock cau be transfected by the necessary genetic information and so after a successful implantation of said cells into the testicles of an acceptor cock a transgenic acceptor cock is so produced, which transgenic acceptor cock after insemination of hens by his ejaculate or by natural breeding will pass ? the genetic information of the donor to his progeny.

A METHOD OF TRANSGENIC FOWL CONSTRUCTION

Field of the Invention

The present invention relates to a method of transgenic fowl construction using germline spermatogonial cells for transfer of genetic information in fowl strain.

Background of the Invention

Biotechnological research in the field of transgenic fowl is far less successful then the one in the field of the mammals. The main cause of this state is the unique reproductive apparatus of the birds.

In difference to the mammals, the bird´s zygote is not accessible and it is connected with an extraordinarily large yolk sac. The yolk mass prevents identification of pronuclei, in the process of fertilization a polyspermia takes place and it is not possible to determine which protonucleus participated in the embryo formation. Therefore, e.g. the direct microinjection into the protonucleus used in case of mice is nearly impossible in case of fowl. Therefore, in the fowl field, activity was directed to manipulation with the not yet differentiated fowl cells of whatever kind, i.e. blastodermal, primordial gonocytes or germline, spermatogonial cells.

Use of germline, spermatogonial cells in construction of transgenic constructs of mice was disclosed at first by Brinster, L.R. and Avarbock, M.R. (1994) Germline Transmission of Donor Haplotype Following Spermatogonial Transplantation, Proc. Natl. Acad. Sci. USA, 91, 11303-11307.

Virtually, it is a new field of possible germline chimaera production consisting in partial or complete destruction of the reproductive cells in testicles of a creature and in a repeated

re-colonisation of the testicles by germ cells of another creature. Application of this method on fowl was not disclosed.

The first step in mastering the above technique is the testicles sterilization of an acceptor because destruction of such germline spermatogonial cells (sperm progenitors) is not easy. The use e.g. of busulphan is not entirely corresponding, and after its use, only partial sterilization of the donor takes place (see Vick, L., Luke, G., Simkiss, K. (1993) Germline Chimaeras Can Produce Both Strains of Fowl with High Efficiency after Partial Sterilization; J. Reprod. Fertil., 98, 637 - 641. On the other side, use of a higher dose brings about a total retardation of the animal what is unwanted in any case (see Smýkalová, S., Kotrbová, A., Trefil, P. (1998) Effect of Busulphan on Growth and Development of the Chicken Embryos, Vet. Med.-Czech, 43, 105-109).
The other necessary step of a successful application of this method is mastering the transfer of the germline spermatogonial cells into the acceptor, i.e. the transfer of those spermatogonial cells that build in functionally and re-colonize testicles of an acceptor, after their transfer, and subsequently produce fertilizing sperms. At this time this brings many difficulties and ambiguous results.

Summary of the Invention

The above mentioned drawbacks are avoided in case of a method of transgenic fowl construction, preferably by transfer of the germline, spermatogonial cells, according to the present invention which method comprises irradiating the testicles only of an acceptor cock with gamma rays repeatedly, whereafter, foreign germline spermatogonial cells of a donor cock are implanted into the undamaged sterile testicles of said acceptor

cock within an interval of 60 days to 2 years after the last irradiation dose of said testicles, whereby, said testicles continue in producing fertilizing sperms that store the whole genetic information of the donor cock. After insemination of hens by the so produced sperms, transgenic fowl chickens are hatched and provided ½ genetic information by said donor cock.

The method according to the present invention is preferably carried out so that the testicles of the acceptor cock are irradiated with gamma rays applying absorbed dose of at least 8 Gy 3-times to 9-times, always within an interval of 3 to 9 days.

Preferably, the method according to the present invention is carried out so that the testicles of the acceptor cock are irradiated with gamma rays using the absorbed dose 8 Gy 5-times in a week interval.

In contradiction to the other disclosed techniques, in the case of this irradiation, the original germline, spermatogonial cells are completely destroyed in the testicles of the acceptor cock, whereafter, the acceptor cook is unable to produce sperms, whereby the testicle structure including the Sertoli's cells remains preserved for the subsequent implantation of foreign germline spermatogonial cells provided by a donor cock. The so implanted germline spermatogonial cells continue in producing sperms that are able to fertilize and that store the genetic information of the germline spermatogonial cells of the donor cock (see the Diagram in Fig. 2).

Production of transgenic fowl by the method according to this invention is easy and provides results to 100 %, because the spermatogonial cells of the donor cock can be transfected by the

required genetic information and so, after a successful implantation of said cells into the testicles of the acceptor cock a transgenic cock is produced, which transgenic cock will transfer to ½ the genetic information to his progeny by insemination or by natural breeding.

The fact itself that this method of transferring germline spermatogonial cells from one creature to another one is functional is extremely valuable and apart from this, it is extremely interesting especially in relation with the creation of transgenic fowl. The germline spermatogonial cells can be easily transfected in vitro by foreign genetic information before the implantation proper, which information will then be transferred to ½ to the offspring in the F1 generation. This will probably open a real way how to use fowl (fowl oviduct) as a bioreactor for production of specific proteins valuable e.g. for the pharmaceutical industry.

Therefore, a great number of tests were carried out to verify the method according to this invention, whereby, some of them are described below as examples with reference to the attached graphs and drawings.

Brief Description of the Drawings

Figure 1 shows a photograph of seminiferous tubulus (Sem.t.) of repeatedly (5-times 8 Gy dose) irradiated of testes of an acceptor cock. Seminiferous epithelium are lined only by Sertoli cells(S.C.) and interstitial tissue consisting of Leydig cells(L.C.). Germline, spermatogonial cells are not visible (PAS stain, enlarged 400-times).

Figure 2 shows a diagram of the method, whereby, a Minorca black cock (ii,EE,b/b) is the donor cock 1, a Leghorn white (II) cock is the acceptor cock 2 which acceptor cock 2 is irradiated by a 8 Gy absorbed dose 5-times to stop production of own sperms. The acceptor cock 2 is then colonized by spermatogonial cells in suspension 4. The acceptor cock then inseminates a Leghorn barred hen 3 (ii,ee,B/-) to produce crossbreeds, i.e. a (ii,Ee,B/b) barred male chicken 5 and a (ii,Ee,b/-) black female chicken 6.

Figure 3 shows a graph of relation between sperms concentration (x1000/ml)(y-axis) and days elapsed after an irradiation (x-axis) 5-times by a 8 Gy absorbed dose.

Figure 4 shows a graph of relation between motility (y-axis) and days (x-axis) elapsed after irradiation 5-times by a 8 Gy absorbed dose.

Figure 5 shows a graph of relation between sperms concentration (x1000/ml)(y-axis) and days elapsed after irradiation (x-axis) by a 18 Gy absorbed dose.

Figure 6 shows a graph of relation between motility (y-axis) and days (x-axis) elapsed after irradiation by a 18 Gy absorbed dose.

Figure 7 shows a graph of relation between sperms concentration (x1000/ml)(y-axis) and days elapsed after irradiation (x-axis) by a 22 Gy absorbed dose.

Figure 8 shows a graph of relation between motility (y-axis) and days elapsed after irradiation (x-axis) by a 22 Gy absorbed dose.

Figure 9 shows a graph of relation between sperms concentration (x1000/ml) (y-axis) and days elapsed after irradiation (x-axis) by a 26 Gy absorbed dose.

Figure 10 shows a graph of relation between motility (y-axis) and days elapsed after irradiation (x-axis) by 26 Gy absorbed dose.

Figure 11 shows a graph of relation between sperms concentration (x1000/ml) (y-axis) and days elapsed after irradiation (x-axis).

Figure 12 shows a graph of relation between motility (y-axis) and days elapsed after irradiation (x-axis).

For the comparison of spermatozoa motility and spermatozoa concentration (from Fig.3 to Fig.12) between treated groups, the linear regression model was used-Baloui(1966), Likeš and Machek(1983). From the slope of on the gradient line it is possible to characterize the changes of the values in the parameters.

Spermatozoa motility of was subjectively estimated according to Trefil, 1995.Selection of chicken sires for semen production in cages. Book of abstracts of the 46th Annual Meeting of the European Association for Animal Production, Prague, September,165. Very good motility has a no. 5 rating and very poor motility no.1.

Statistically, there was no difference between the control group (Fig.12) and the groups of cocks irradiated with 18 Gy (Fig.6) and 22 Gy (Fig.8). Groups treated with a single dose of 26 Gy (Fig.10) and 5 x 8 Gy (Fig.4) doses differed significantly in their linear gradients compared with the control group and those administered with 18 Gy and 22 Gy.

From a statistical point of view, the linear gradients of the spermatozoa concentration did not differ between the control group (Fig.11) and groups irradiated with single 18 (Fig.5) and 22Gy (Fig.7) doses. However, a significant difference was shown with the single 26Gy group (Fig.9) and the group repeatedly irradiated by 5 doses of 8Gy (Fig.3) compared to 18Gy, 22Gy and control groups.

The group treated with one dose of 26Gy never attained zero spermatozoa concentrations.

Detailed Description of the Invention

Example 1

Complete sterilization of an acceptor cock:

A mature cock of the Leghorn white inbred strain (white colour of feathers is caused by the dominant allele II in locus I) producing ejaculate was fixed in a box so that it was possible to carry out an irradiation with gamma rays directed on the side of his body exactly to an area of the testicle´s size. The

instrument, model Theratron T1000,with isotope ^{60}Co was suitable for this purpose. Using this instrument testicles of this cock were irradiated 5-times in a week minimally by the absorbed dose 8 Gy. Without producing any adverse effects on the cock health and breeding condition, about 90 days after the last irradiation the cock lost the ability to produce sperms permanently and after this period his testicles were ready to be colonized by the germline spermatogonial cells produced by the donor cock, see the graphs in Fig. 3 and Fig. 4 and the photograph in Fig. 2.

Preparation and application of germline spermatogonial cells of the donor cock:

A mature cock of the Minorca black inbred strain (black color of feathers is caused by the recessive allele ii) producing ejaculate was subjected to a tissue sampling by biopsy from his testicles which sample contained his germline spermatogonial cells. The cells were immersed into the M 199 Sigma medium (containing expressed by weight: 10 % of fetal bovine serum, 2 % of chicken serum, 1 % of pyruvate sodium, 1 % of gentamycin), whereby, the dilution ratio with this medium was 1:1. Cell incubation was carried out in standard conditions, i.e. carbon dioxide content 5 % by weight, temperature 40 °C. Spermatogonial cells could be transfected with any foreign DNA during this incubation. Thereafter, the total volume 200 microlitres of the germline spermatogonial cells suspension was applied by means of a syringe into each irradiated testicle of the acceptor cock at the time when the irradiated cock ceased to produce any ejaculate, i.e. 85 to 100 days after the last irradiation dose.

Functional production of ejaculate after a transfer of the germline spermatogonial cells of the donor cock:

The irradiated acceptor cock of the Leghorn inbred strain with non-functional testicles started to produce ejaculate after application of foreign germline spermatogonial cells of the donor cock of the Minorca black inbred strain.

Biological testing of the transferred germline spermatogonial cells of the donor cock:
Having carried out insemination of a hen of the Leghorn barred inbred strain (the barred color is caused by recessive allele ii by piebald gene bound to sex B/-) by ejaculate taken from the irradiated cook of the Leghorn white inbred strain, which cock is acceptor of foreign germline spermatogonial cells of the donor cock of the Minorca black inbred strain, the inseminated eggs were put into an incubator and after 21 days of incubating only black chicken hatched.
The black chicken are a sound proof that the implantation and colonization of the germline spermatogonial cells of the donor cock was successful because if only black or barred chicken hatch, in fact it indicates that the dominant allele II is not present and that only the recessive allele ii is present, i.e. that only functional sperms of the cock with recessive feather color ii are present, i.e. in this case of those with the black feather color, see Fig. 2.

Example 2
Incomplete sterilization of the acceptor cock:
A mature cock of the Leghorn white inbred strain (white feather colour caused by dominant allele II in locus I) producing ejaculate was fixed for irradiation in the same way as in Example 1. Testicles of this cock were irradiated by means of the irradiation instrument by a one-time absorbed dose of 18 Gy. Without deteriorating the health state and the breeding condition of the cock, the cock did not loose the ability to

produce sperms permanently (in comparison to check, see the graphs in Figures 11 and 12), on the contrary, after 150 days the concentration (see the graph in Fig. 5) and the motility (see the graph in Fig. 6) returned to normal values before the irradiation and the cock testicles were not prepared to be colonized by germline spermatogonial cells of the donor cock.

Example 3

Incomplete sterilization of an acceptor cock:

A mature cock of the Leghorn white inbred strain (white colour of feathers is caused by the dominant allele II in locus I) producing ejaculate was fixed in a box so as in Example 1 during irradiation. Testicles of this cock were irradiated with a one-time absorbed dose 22 Gy provided by the same irradiation instrument.

Without producing any adverse effects on the cock's health and breed condition, during 200 days of monitoring, the cock did not lose the ability to produce sperms permanently. Conversely, after 160 days, the concentration (see graph in Fig. 7) and motility (graph in Fig. 8) were a little bit lower, but they returned to the original level existing before the irradiation and the cock´s testicles were not prepared to be colonized by germline spermatogonial cells of the donor cock.

Example 4

Incomplete sterilization of the acceptor cock:

A mature cock of the Leghorn white inbred strain (white color of feathers is caused by the dominant allele II in locus I) producing ejaculate was fixed in a box so as in Example 1 during irradiation. Testicles of this cock were irradiated with a one-time absorbed dose 26 Gy provided by the same irradiation instrument. This absorbed dose influenced health state of this cock negatively. The cock´s skin was slightly swollen and

reddish in the irradiation place, the cock was tired for several days after the irradiation. The cock lost ability to produce sperms not sooner then nearly after 160 days, (see graph in Fig. 9), but production of ejaculate continued at a very low level and the sperms have shown certain motility (see graph in Fig. 10). It was obvious that this absorbed dose was rather too high, it deteriorated health state of the cock, but considering that the ejaculate production continued, this absorbed dose was still insufficient and the cock testicles were not yet prepared for colonization by the germline spermatogonial cells of the donor cock.

Industrial Use

By repeated irradiation of the acceptor cock´s testicles with gamma rays and by implanting foreign germline spermatogonial cells of the donor cock into the so treated undamaged but sterile testicles of the acceptor cock, sperms able to fertilize that store all genetic information of the donor cock are repeatedly produced by this acceptor cock. After insemination of hens by the sperms so generated, transgenic fowl is produced which fowl is provided to ½ with the genetic information of the donor cock.

Method according to the present invention will find industrial use in fowl breeding and biotechnological industries.

Production of transgenic fowl by this method is easy because the spermatogonial cells of the donor cock can be transfected by the necessary genetic information and so after a successful implantation of said cells into testicles of a acceptor cock a transgenic cock is so produced, which cock after insemination of

hens by his ejaculate or by natural breeding will pass to ½ the genetic information of the donor to his offspring.

Claims

1. A method of transgenic fowl construction using germline spermatogonial cells for transfer of genetic information in fowl strain, characterized in that the testicles only of an acceptor cock are irradiated with gamma rays repeatedly and externally, whereafter, the so treated preserved but now sterile testicles of this acceptor cock are colonized by foreign germline spermatogonial cells of a donor cock, which cells then produce sperms that store all genetic information of the donor cock, whereby, the sperms are able to fertilize hens after their insemination, whereby, transgenic fowl offsprings provided to ½ with the donor cock genetic information are breed.

2. A method according to Claim 1 characterized in that said testicles of said acceptor cock are irradiated with gamma rays up to the absorbed dose of at least 8 Gy, what is carried out 3-times to 9-times, always in 3 to 9 day intervals.

3. A method according to Claim 1 characterized in that said testicles of said acceptor cook are irradiated with gamma rays up to the absorbed dose 8 Gy always in week intervals.

A photography of the seed producing ducts after repeated (5-times 8 Gy dose) irradiation of testicles of an acceptor cock. In the sperm producing ducts only the Sertoli's cells are visible, the germline spermatogonial cells are not visible (dyed by Pas, enlarged 400-times).

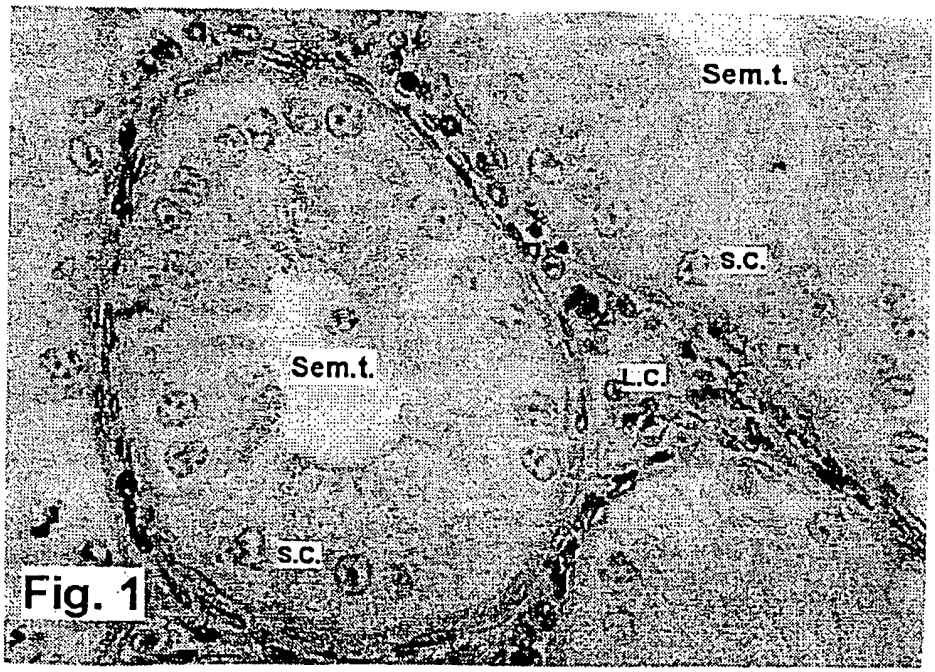


Fig. 1

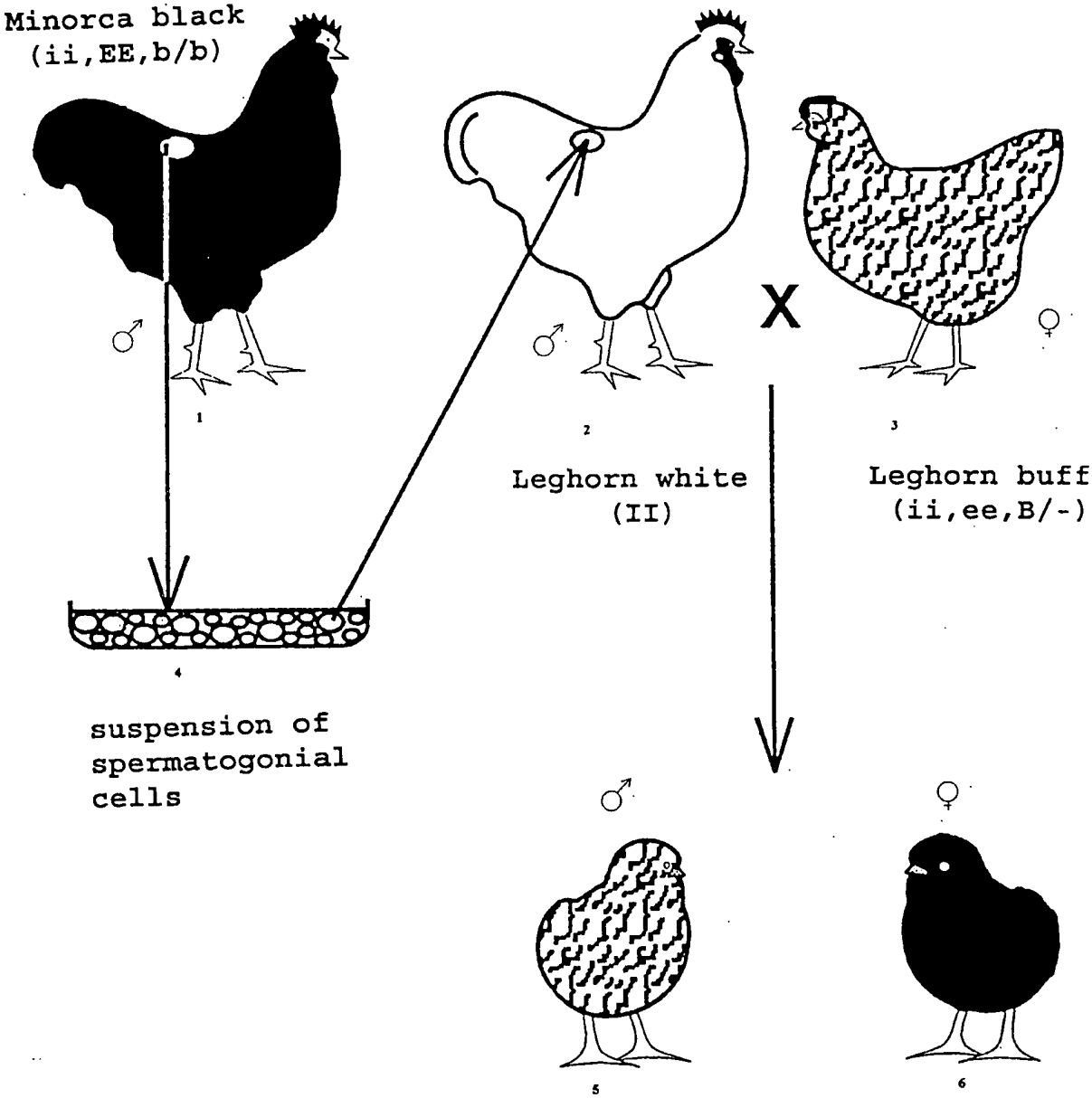

Minorca black
(ii,EE,b/b)
♂
1
♂
2
X
♀
3
Leghorn white
(II)
Leghorn buff
(ii,ee,B/-)
4
suspension of
spermatogonial
cells
♂
5
♀
6
(ii,Ee,B/b)
(ii,Ee,b/-)

Fig. 2

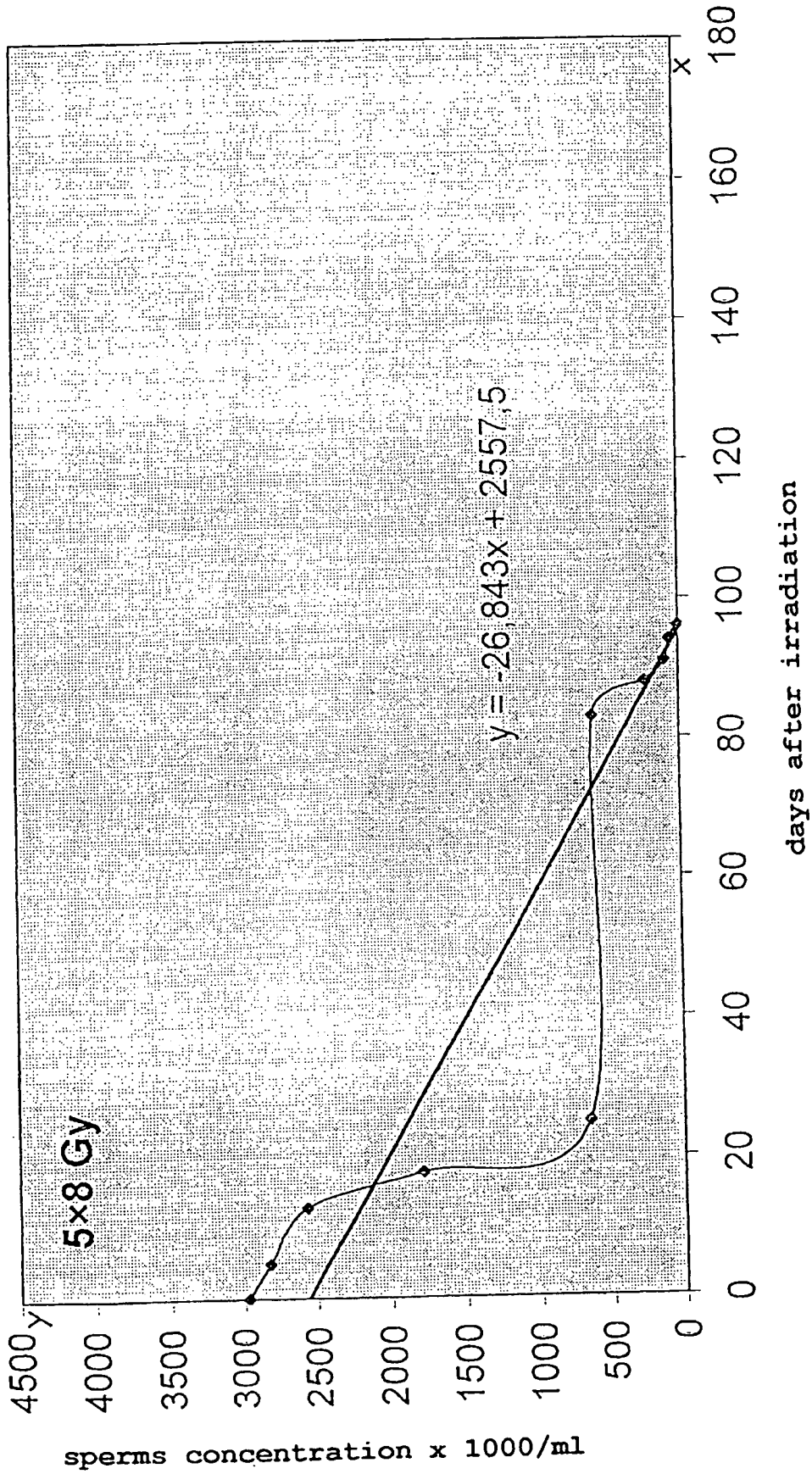

5x8 Gy
y = -26,843x + 2557,5
y
x
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
140
160
180
sperms concentration x 1000/ml
days after irradiation

Fig. 3

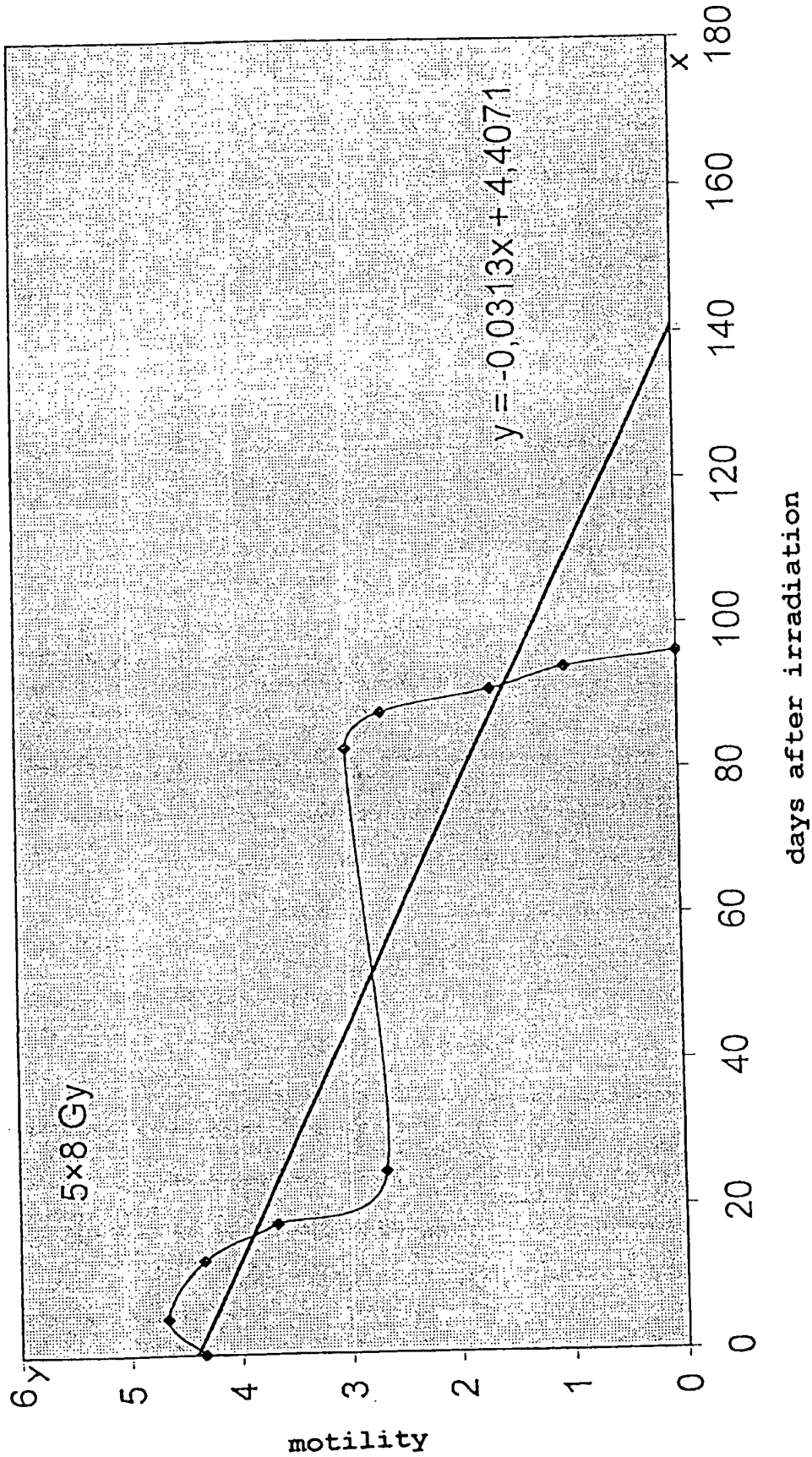

y = -0,0313x + 4,4071
5x8 Gy
motility
days after irradiation
y
x
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180

Fig. 4

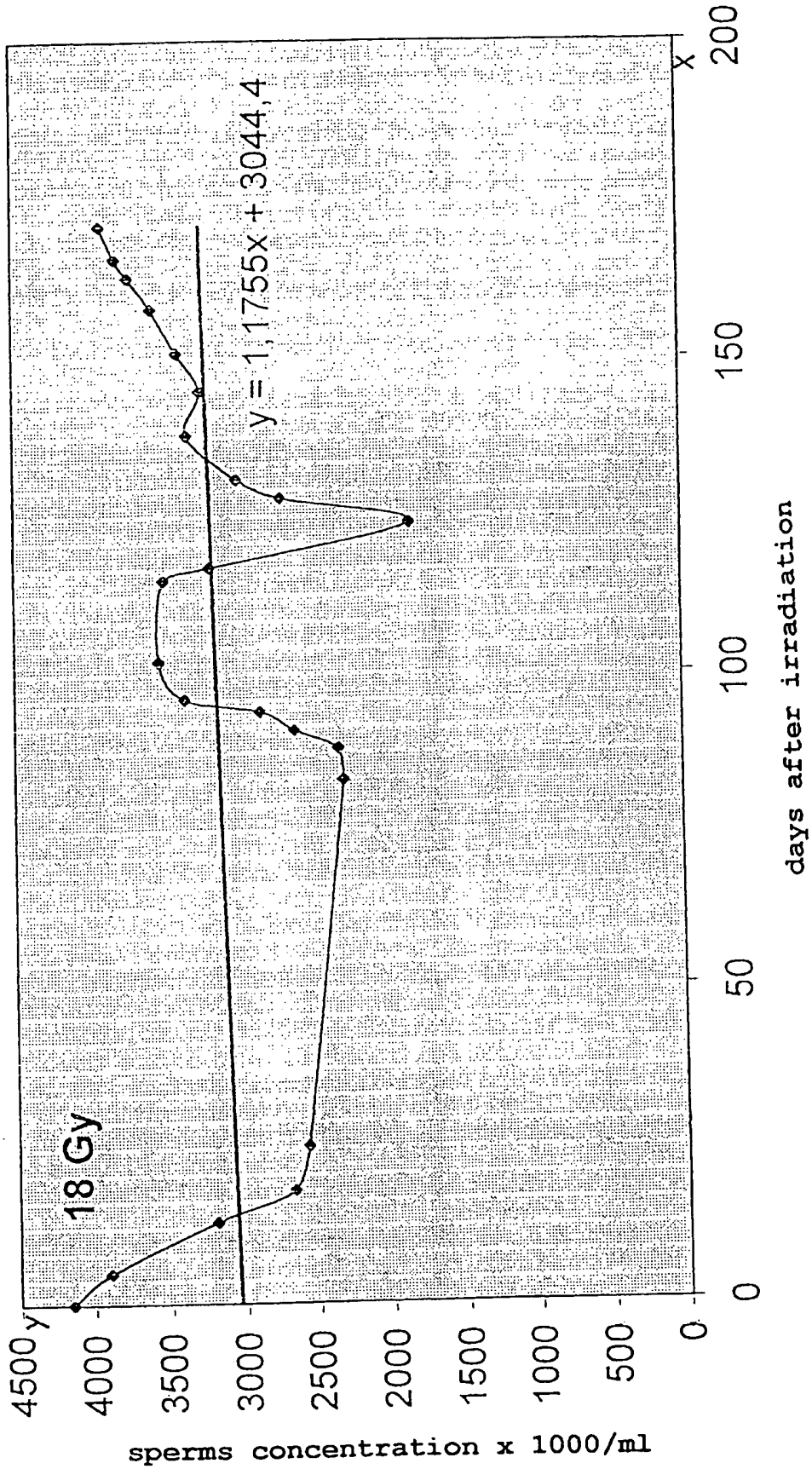

18 Gy
y = 1,1755x + 3044,4
sperms concentration x 1000/ml
days after irradiation
4500 y
4000
3500
3000
2500
2000
1500
1000
500
0
0
50
100
150
200
x

Fig. 5

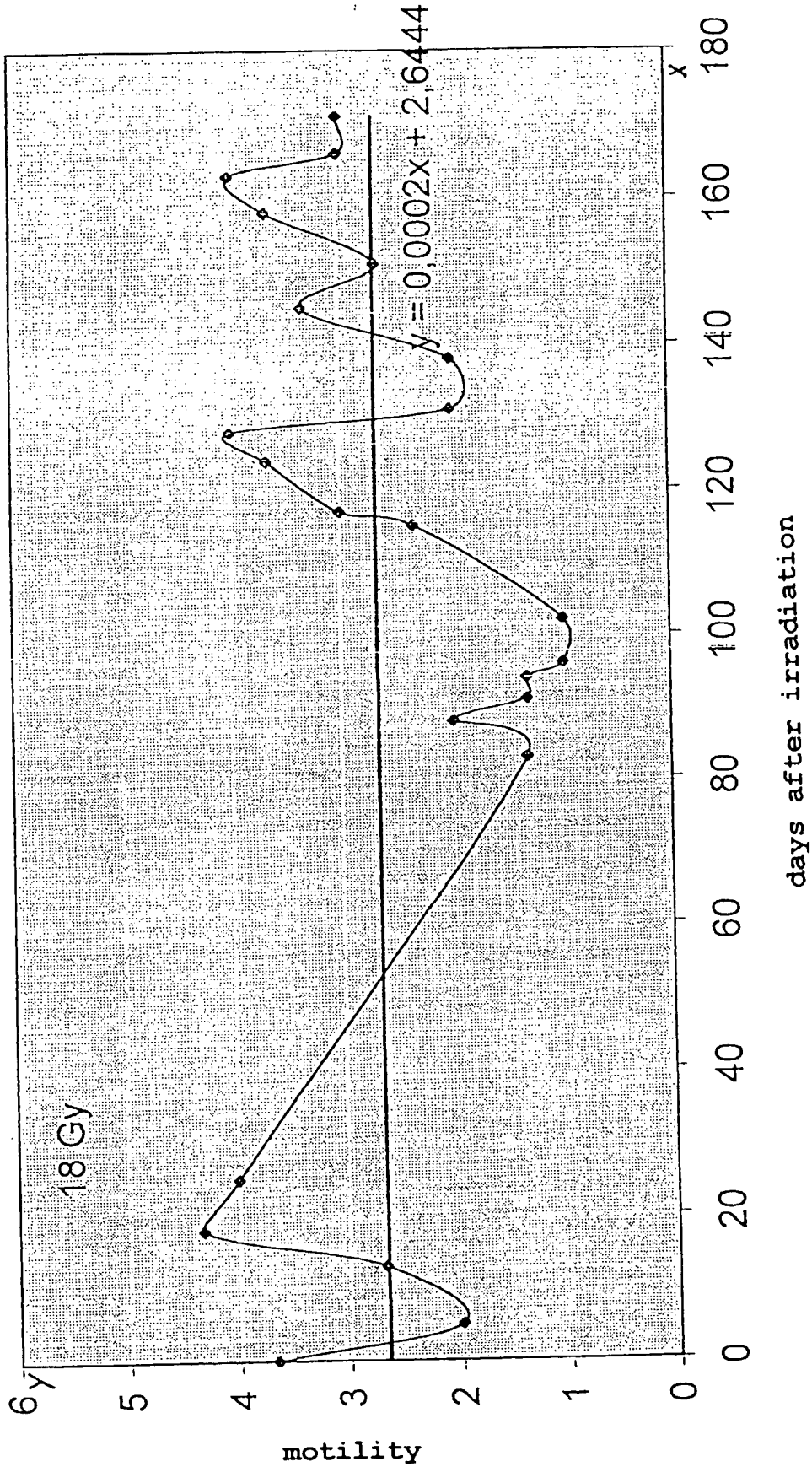

y = 0,0002x + 2,6444
18 Gy
motility
days after irradiation
y
x
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180

Fig. 6

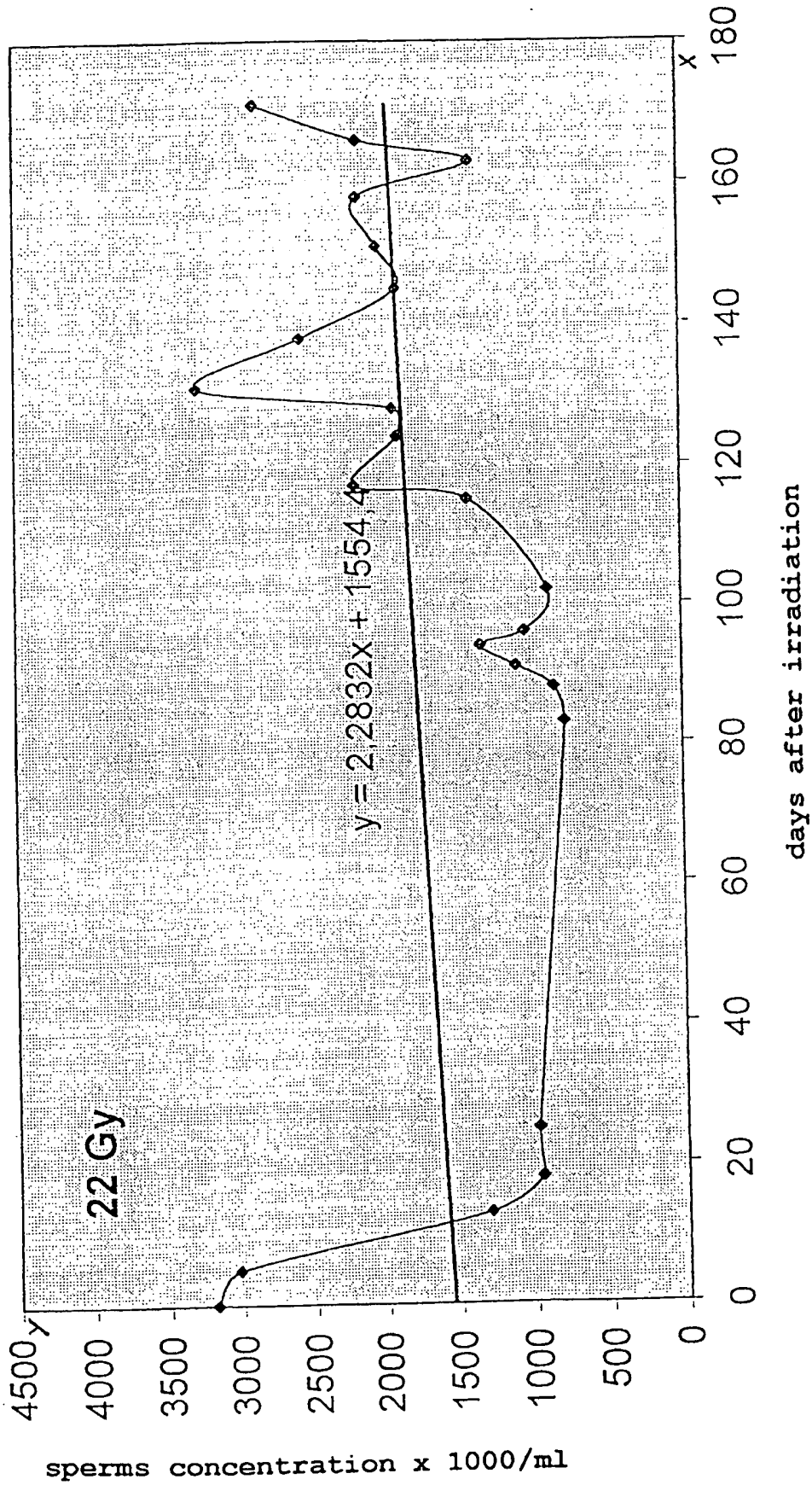

22 Gy
y = 2,2832x + 1554,4
sperms concentration x 1000/ml
days after irradiation
y
x
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
140
160
180

Fig. 7

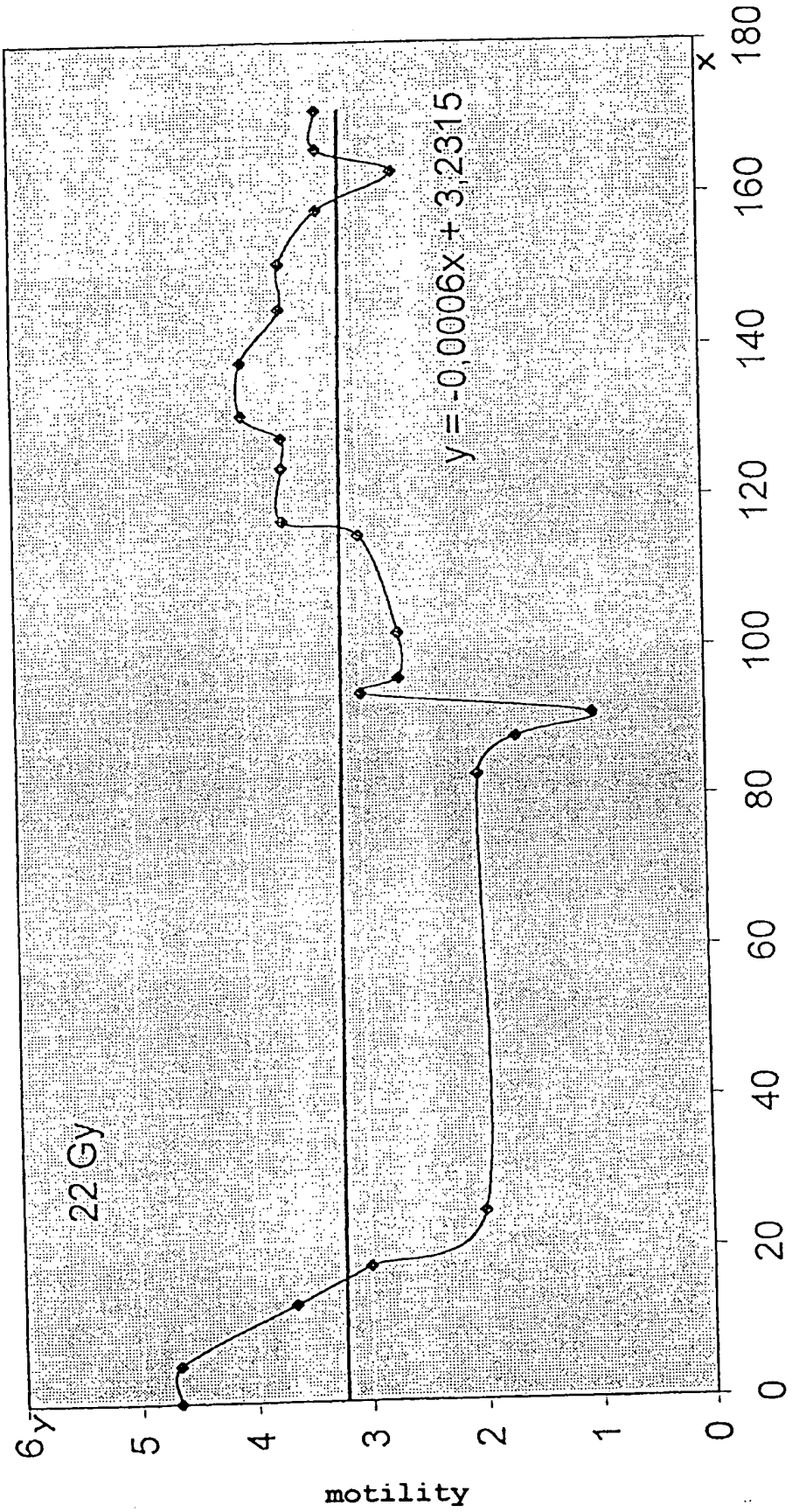

y = -0,0006x + 3,2315
22 Gy
6y
5
4
3
2
1
0
motility
0
20
40
60
80
100
120
140
160
180
x
days after irradiation

Fig. 8

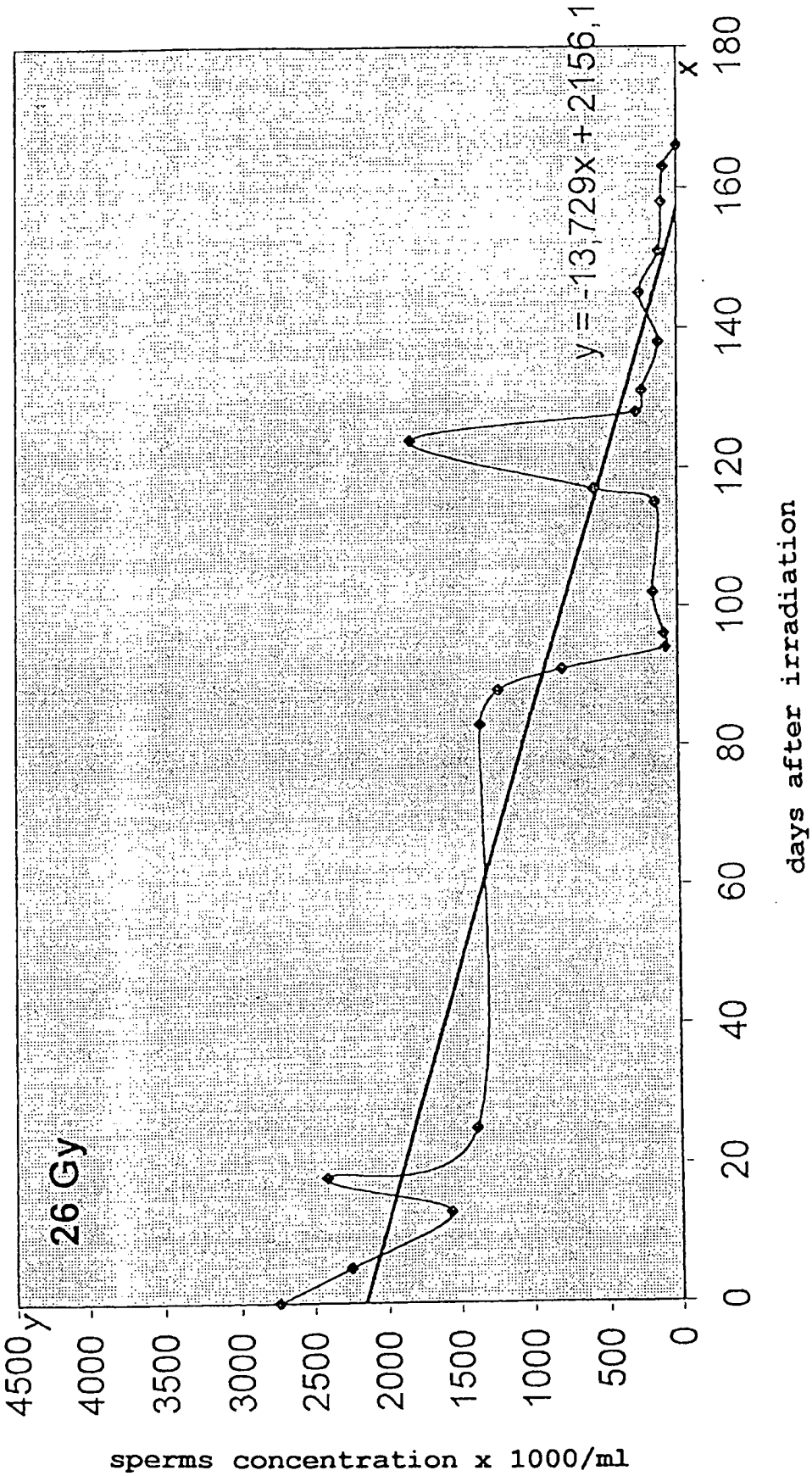

26 Gy
y = -13,729x + 2156,1
y
x
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
140
160
180
sperms concentration x 1000/ml
days after irradiation

Fig. 9

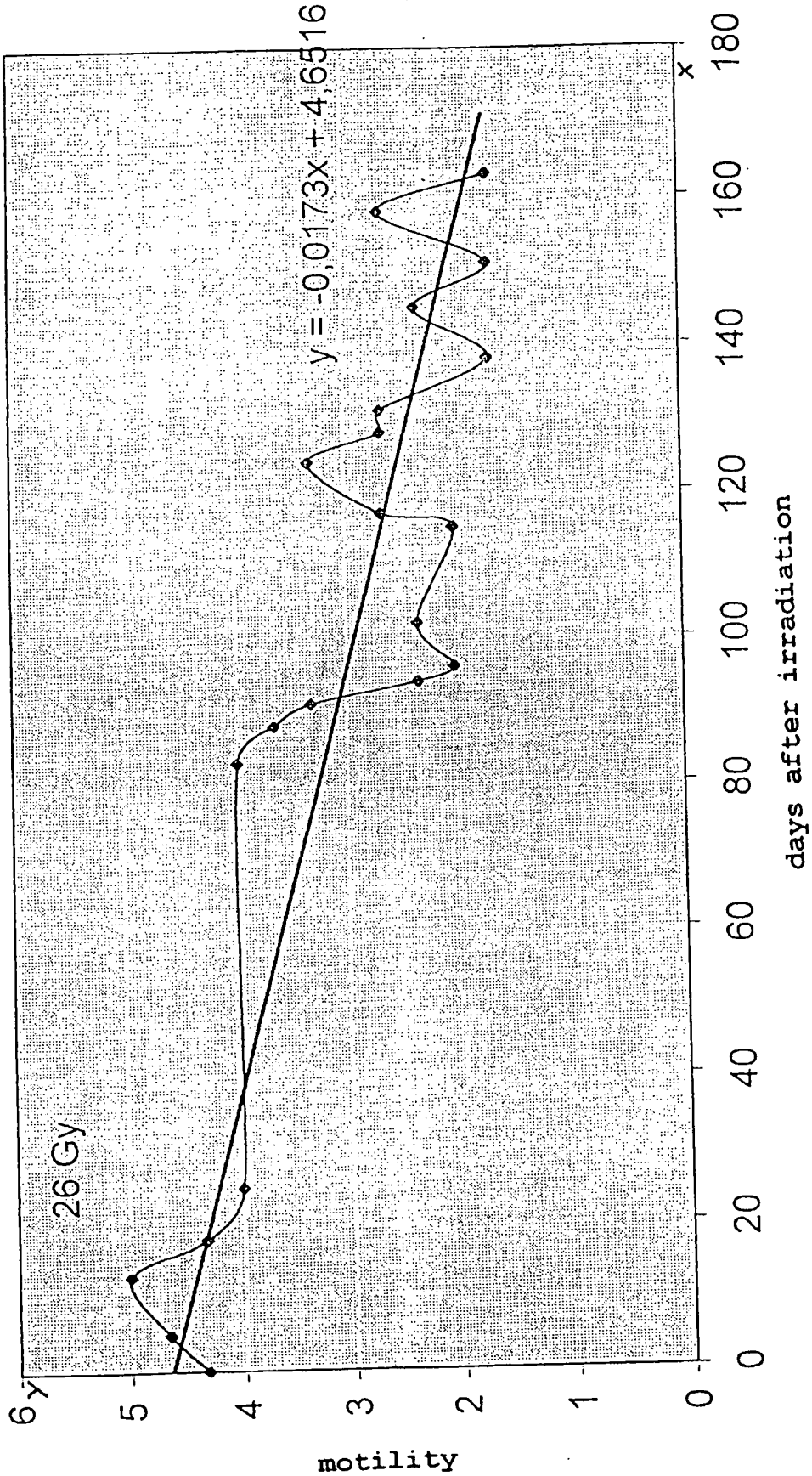

y = -0,0173x + 4,6516
26 Gy
motility
days after irradiation
y
x
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180

Fig. 10

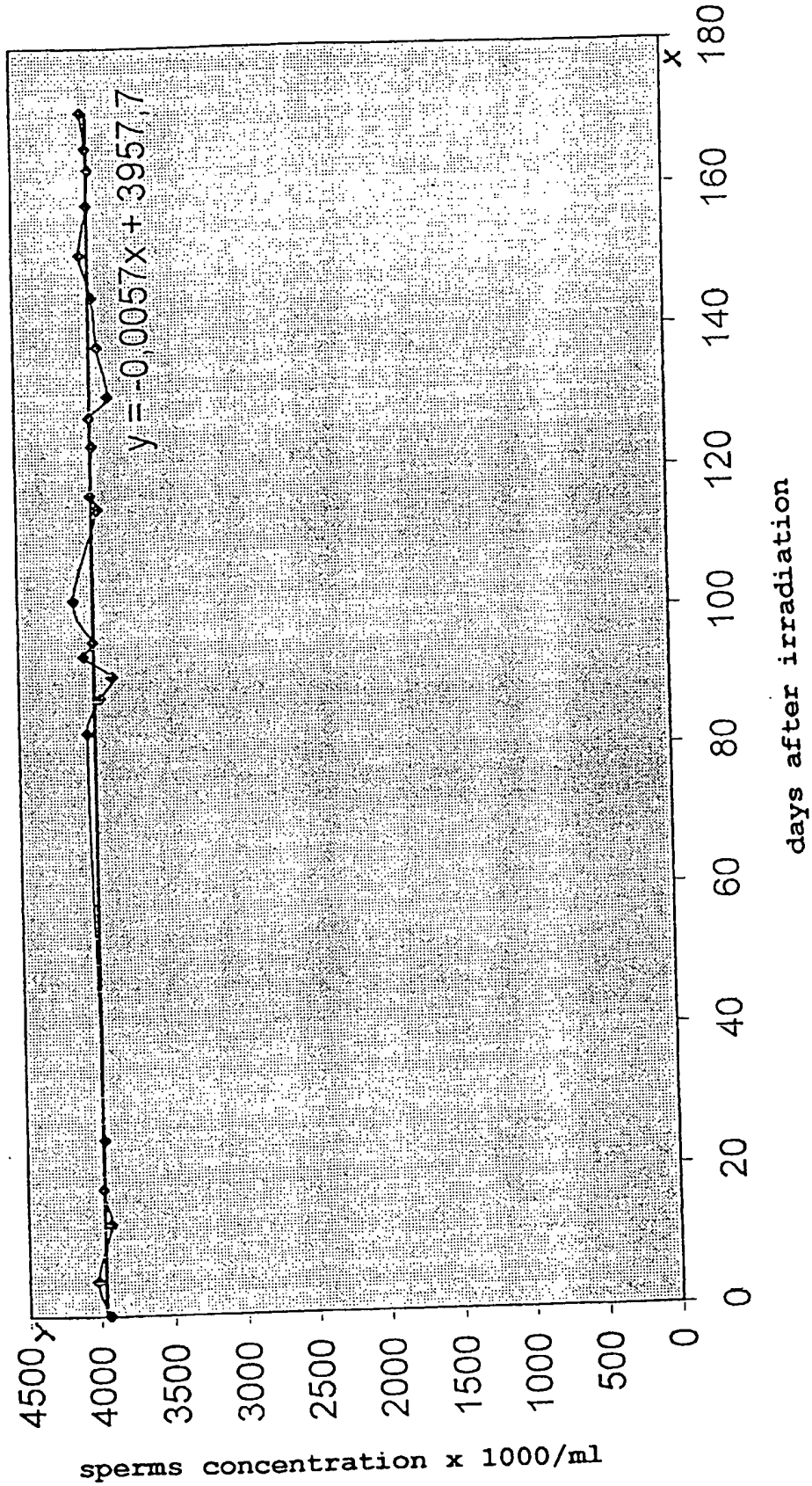

y = -0,0057x + 3957,7
sperms concentration x 1000/ml
days after irradiation
y
x
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
140
160
180

Fig. 11

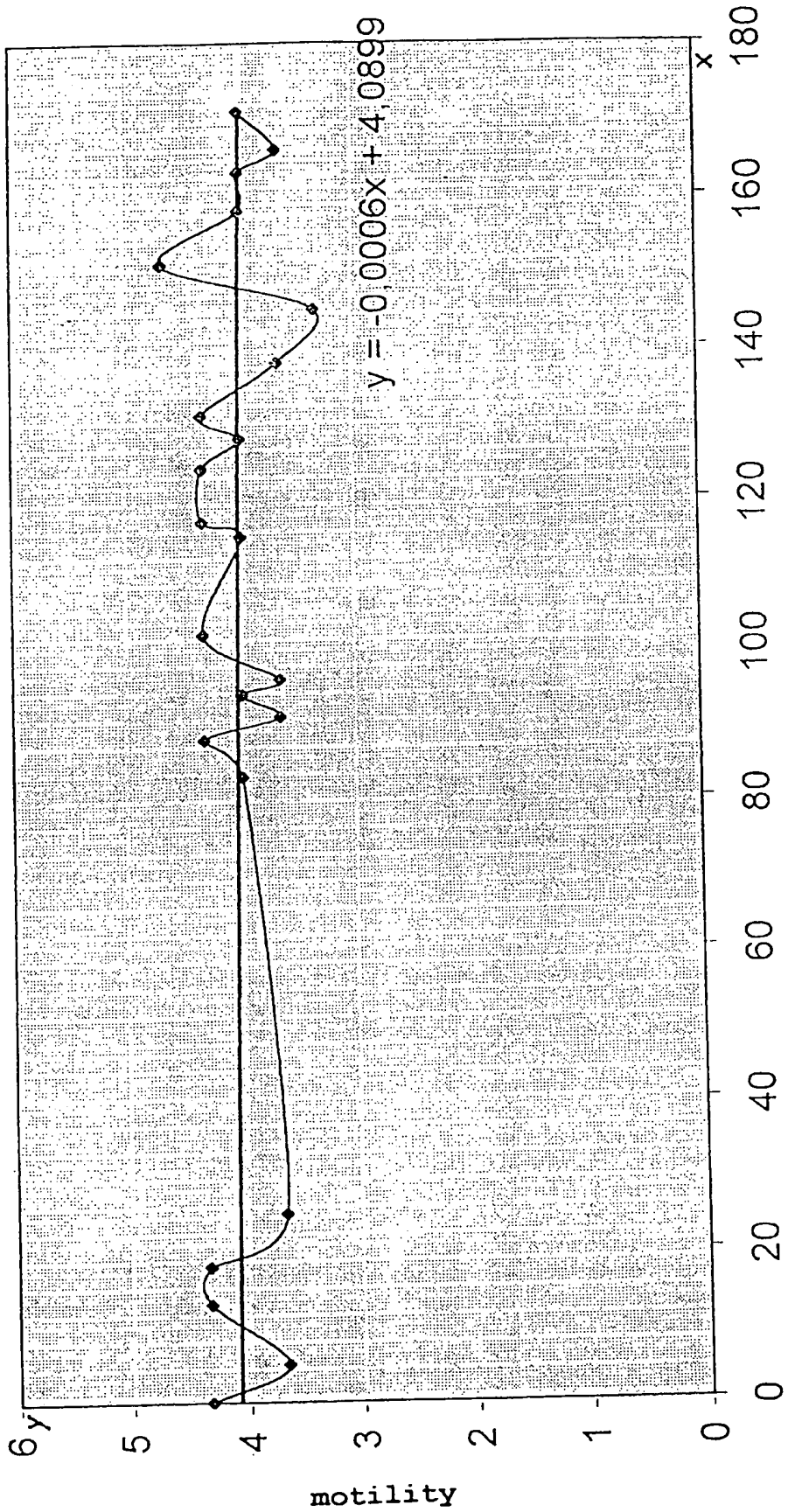

y = -0,0006x + 4,0899
motility
days after irradiation
0
1
2
3
4
5
6
y
0
20
40
60
80
100
120
140
160
180
x

Fig. 12

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/17344 A3

(51) International Patent Classification[7]: A01K 67/027

(21) International Application Number: PCT/CZ00/00064

(22) International Filing Date:
8 September 2000 (08.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PV 3186-99 8 September 1999 (08.09.1999) CZ

(71) Applicant *(for all designated States except US)*: **BIOPHARM, VÝZKUMNÝ ÚSTAV BIOFARMACIE A VETERINÁRNÍ CH LÉČIV A.S.** [CZ/CZ]; Pohoří-Chotouň, 254 49 Jílové u Prahy (CZ).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **TREFIL, Pavel** [CZ/CZ]; K Netlukám 962, 104 00 Praha 10 (CZ). **KOTRBOVA, Alena** [CZ/CZ]; Peškova 729, 341 01 Horaždovice (CZ).

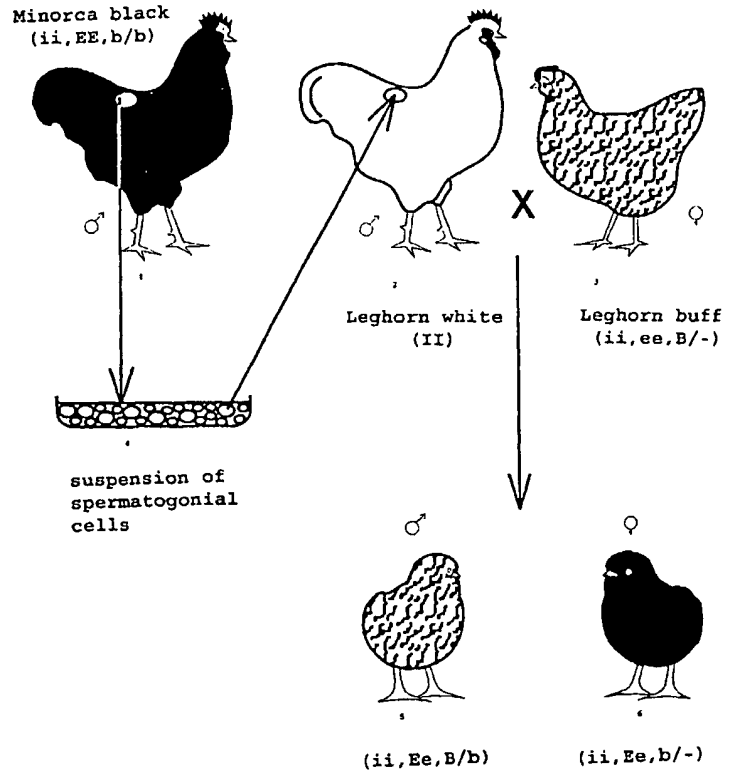
(74) Agent: **PATENTSERVIS PRAHA A.S.**; Jívenská 1/1273, 140 21 Praha 4 (CZ).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

[Continued on next page]

(54) Title: A METHOD OF TRANSGENIC FOWL CONSTRUCTION



(57) Abstract: A method of transgenic fowl construction using germline spermatogonial cells for transfer of genetic information in fowl strain, which method is carried out so that the testicles only of an acceptor cock are irradiated with gamma rays up to the absorbed dose 8 Gy in one irradiation repeatedly and externally. Thereby, the original germline spermatogonial cells in the testicles of the acceptor cock are destroyed, which acceptor cock is then not able to produce sperms, whereby, the testicles structure, including the Sertoli's and Leydig's cells remains preserved for implantation of foreign germline spermatogonial cells of a donor cock. The new implanted germline spermatogonial cells then continue in production of sperms. The sperms are able to fertilize and that store all genetic information of the germline spermatogonial cells of the donor cock. The implantation is carried out after 50 days and more after the last irradiation.

WO 01/17344 A3

Published:
— *with international search report*

(88) Date of publication of the international search report:
10 January 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/CZ 00/00064

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BENOIT J ET AL: "GREFFES INTERRACIALES DE TESTICULES CHEZ LES GALLINACES. TESTOTESTES FERTILES OBTENUS PAR GREFFE DE TESTICULES DE POUSSINSWYANDOTTE BLANC DANS LES TESTICULES STERILISES PAR LES RAYONS X DE COQS RHODE ISLAND RED" COMPTES RENDUS DES SEANCES DE LA SOCIETE DE BIOLOGIE ET DE SES FILIALES, XX, XX, vol. 162, no. 4, 23 November 1968 (1968-11-23), pages 838-842, XP000998009 ISSN: 0037-9026 page 839, paragraph 4 -page 840 --- -/--	1

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 August 2001	03/09/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chambonnet, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/CZ 00/00064

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	BRINSTER R L ET AL: "SPERMATOGENESIS FOLLOWING MALE GERM-CELL TRANSPLANTATION" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 91, November 1994 (1994-11), pages 11298-11302, XP000993002 ISSN: 0027-8424 the whole document ---	1
A	BRINSTER R L ET AL: "GERMLINE TRANSMISSION OF DONOR HAPLOTYPE FOLLOWING SPERMATOGONIAL TRANSPLANTATION" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 91, November 1994 (1994-11), pages 11303-11307, XP000993003 ISSN: 0027-8424 cited in the application the whole document ---	1
A	AIGE-GIL V; SIMKISS K : "STERILIZATION OF AVIAN EMBRYOS WITH BUSULPHAN" RESEARCH IN VETERINARY SCIENCE , vol. 50, 1991, pages 139-144, XP000998022 cited in the application the whole document ---	1
A	VICK L ET AL: "GERM-LINE CHIMERAS CAN PRODUCE BOTH STRAINS OF FOWL WITH HIGH EFFICIENCY AFTER PARTIAL STERILIZATION" JOURNAL OF REPRODUCTION AND FERTILITY, JOURNALS OF REPRODUCTION AND FERTILITY LTD, GB, vol. 98, no. 2, July 1993 (1993-07), pages 637-641, XP000998004 ISSN: 0022-4251 the whole document ---	1
A	AIGE-GIL V ET AL: "STERILISING EMBRYOS FOR TRANSGENIC CHIMAERAS" BRITISH POULTRY SCIENCE, LONGMAN GROUP, GB, vol. 32, 1991, pages 427-438, XP000994670 ISSN: 0007-1668 the whole document --- -/--	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/CZ 00/00064

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WISHART G J ET AL: "EFFECT OF GAMMA-RADIATION ON FOWL SPERM FUNCTION IN VITRO AND IN VIVO" JOURNAL OF REPRODUCTION AND FERTILITY, JOURNALS OF REPRODUCTION AND FERTILITY LTD, GB, vol. 75, no. 2, November 1985 (1985-11), pages 617-622, XP000998034 ISSN: 0022-4251 the whole document ---	1
A	VICK L ET AL: "TRANSGENIC BIRDS FROM TRANSFORMED PRIMORDIAL GERM CELLS" PROCEEDINGS OF THE ROYAL SOCIETY OF EDINBURGH. SECTION B, BIOLOGICAL SCIENCES, ROYAL SOCIETY OF EDINBURGH, EDINBURGH, GB, vol. 251, no. 1332, 22 March 1993 (1993-03-22), pages 179-182, XP000997980 ISSN: 0269-7270 page 182, column 1, paragraph 2 ------	1

1